many embodiments an sdABD(½) as defined herein, although as discussed herein, this is optional and/or can be replaced by other half-life extension domains; additionally, the half-life extension domain can also be N-terminal to the construct or internal as well. Figure 4 also has the VH and VL of the Fv and iVH and iVL of the pseudo Fv in a specific order, e.g. from N- to C-terminal, VH-linker-VL (and iVL-linker-iVH) although as will be appreciated by those in the art, these can be reversed (VL-linker-VH and iVH-linker-iVL). Alternatively, one of these Fvs can be in one orientation and the other in the other orientation, although the expression of protein in the orientation as shown here was surprisingly higher than the other orientations.

[0028] Figure 5A – Figure 5G depict a number of sequences of the invention. For antigen binding domains, the CDRs are underlined. As is more fully outlined herein, these domains can be assembled in a wide variety of configurations in the present invention, including "format 1", "format 2", "format 3" and "format 4" orientations. Of note is that SEQ ID NO:90 is cleaved by MMP9 slightly faster than SEQ ID NO:s 75 and 76, and SEQ ID NO:91 is cleaved slower than SEQ ID NO:s 75 and 76.

[0029] Figure 6A – Figure 6B depict a number of suitable protease cleavage sites. As will be appreciated by those in the art, these cleavage sites can be used as cleavable linkers. In some embodiments, for example when more flexible cleavable linkers are required, there can be additional amino acids (generally glycines and serines) that are either or both N- and C-terminal to these cleavage sites.

[0030] Figure 7A – Figure 7D depict some data associated with the "Format 3" or "hemi-COBRA™" structures. This shows that Format 3 constructs bind co-operatively to CD3 after cleavage by protease (in this case EK protease, although any of the protease cleavage sites outlined herein and depicted in Figure 5 and Figure 6 can be used) and create a CD3 binding site, as shown by a sandwich FACS analysis.

[0031] Figure 8A – Figure 8D shows that the protease cleavage co-operatively activates T-cell killing of EGFR+ target cells with complimentary hemi-COBRA™ pairs. Figure 8A and Figure 8B show that the constructs in isolation, but cleaved with different concentrations of protease, do not affect target cell viability. However, Figure 8C shows that in combination, in the presence of protease, target cell viability is significantly diminished. Figure 8D shows the general mechanism.

[0032] Figure 9 shows some non-target controls for use in the assays to test efficacy of the Format 1 constructs.

[0033] Figure 10A – Figure 10F shows the generation of an active CD3 binding domain is dependent on target binding of both "arms", e.g. the sdABD-TTA domains, one of which is on each of the two constructs. The TDCC assay was done as described in the Examples.

[0034] Figure 11 shows the schematic of suitable hemi-COBRA™ pairs. "Mep" stands for a meprin protease cleavage site, "His-6" is a tag as more fully discussed herein, ST14 is a matriptase protease cleavage site and "Thb" is a thrombin protease cleavage site.

[0035] Figure 12A – Figure 12C shows the TDCC data associated with the constructs of Figure 11. Figure 12A shows that addition of pre-cleaved hemi-COBRA pairs results in efficacy on OvCAR8 cells, Figure 12B shows that addition of pre-cleaved hemi-COBRA pairs results in efficacy on HCT116 cells, and Figure 12C shows that addition of pre-cleaved hemi-COBRA pairs results in efficacy on LoVo cells, all of which are cancer cell lines.

[0036] Figure 13A – Figure 13B shows that the MMP9 linker is stable in vivo. NSG mice were administered a single intravenous bolus dose of either Pro40 (MMP9 cleavable), Pro74 (non-cleavable) via the tail vein at a dose level of 0.5 mg/kg. The dose solution for each compound was prepared in a vehicle of 25mM citric acid, 75-mM L-arginine, 75mM NaCl and 4% sucrose pH 7.0. Two blood samples were collected at preselected times from each animal, one towards the beginning of the study, collected by orbital bleed or submandibular bleed, and another at the terminal time point by cardiac puncture. The time points for blood collection were 0.083, 1, 6, 24, 72, and 168h. Plasma was prepared from each individual blood sample using K_2 EDTA tubes. Concentrations were determined using an MSD assay with a MAb specific to the anti-HSA sdABD and detected with the EGFR extracellular domain.

[0037] Figure 14 depicts the schematics of the Format 3A hemi-COBRA™ constructs used in the experiments depicted in Figure 15. Pro51 is the positive control, as it is "always on", since it forms an active anti-CD3 Fv. Pro98 is a negative control, since it's sdABD is directed against hen egg lysozyme, which isn't expressed by the tumor. Pro77 and Pro53 are the pro-drug Format 3A pair, using sdABDs against EGFR and an MMP9 cleavage site. Pro74 and Pro72 is a negative control Format 3A pair, since they don't have cleavage sites.

[0038] Figure 15 shows that Format 1 constructs work to regress tumors in vivo, using two different tumor cell lines implanted into mice using the protocols in the Examples. Anti-tumor activity with the hemi-COBRA constructs (Pro77 and Pro53) was dependent on the inclusion of both the anti-EGFR sdABDs and the MMP9 cleavable linkers, along with the active anti-CD3 Fv.

[0039] Figure 16 shows the schematic of the next generation format, a full length construct that has two pseudo Fv domains with cleavable sites between them, as is generally described in US 2018/0134789, hereby incorporated by reference. However, as shown in the following figures, this first generation full length construct does not show very good conditionality, as it can isomerize to form both an active and inactive construct.

[0040] Figure 17 shows that the Format 3A construct pairs actually show better conditionality than the Pro100 first generation full length constructs.

[0041] Figure 18 depicts additional first generation full length constructs that were tested in Figure 19.

[0042] Figure 19 shows that the first generation constructs show high activity even in the uncleaved format, e.g. poor conditionality.

[0043] Figure 20 shows that the first generation full length constructs show two monomer peaks on analytical SEC.

[0044] Figure 21 shows the schematic of the reason for the noncleaved activity, which is that the full length first generation constructs isomerize to form two conformations, one which is inactive since there is no active anti-CD3 Fv formed (the "bivalent scFv"), and the other which is active in the absence of protease, a "single chain diabody" type of configuration. See PEDS 23(8):667-677 (2010).

[0045] Figure 22 shows the results of a TDCC assay, run at 37C for 2 days, with the first generation single chain constructs. The results show that the uncleaved constructs show strong killing. These results led to the generation of the Format 1 constructs.

[0046] Figure 23A - Figure 23G shows Format 1 constructs used in the invention. As will be appreciated by those in the art and described herein, these are depicted with a sdABD-EGFR targeting moiety, although sdABDs to other TTAs can be used.

[0047] Figure 24 shows that the Format 1 constructs (Pro140 in this case) form a single isomer that is stable at 37C.

[0048] Figure 25 depicts that Format 1 constructs have very low binding to human CD3 in the uncleaved format, as measured by an Octet assay. The top line is Pro120, the middle line is Pro51 (the positive control) and the bottom lines are Pro140 held at either 4C or 37C for 3 days.

[0049] Figure 26 similarly depicts that the Format 1 constructs have very low TDCC activity in the uncleaved form.

[0050] Figure 27 depicts a specific Format 1 construct, Pro140, used in *in vivo* testing, using sdABD-EGFR as the targeting moieties and an MMP9 cleavage site.

[0051] Figure 28A – Figure 28B shows tumor regression using a Format 1 construct.

[0052] Figure 29 depicts that due to the cleavage site in the constrained Fv, several different fragments can be generated: a partially cleaved fragment and the fully cleaved fragment. Surprisingly, the partially cleaved format is more active than the fully cleaved format, leading to the generation of Format 2.

[0053] Figure 30 shows a number of Format 2 schematics, all of which use sdABD-EGFR targeting domains, although as outlined herein and listed in the sequences, sdABDs to other TTAs can be used. Pro51 and Pro201 are positive controls (in an active "hemi" configuration), and Pro214 is a full length negative control, as there is no cleavage site.

[0054] Figure 31 shows the TDCC activity of Format 2 construct Pro187, which uses a meprin cleavage site. Pro187 in the TDCC assay was 1200-fold more active when added pre-cleaved than when added uncleaved. The pre-cleaved Pro187 demonstrated activity that fell between the positive controls Pro51 and Pro201. The uncleaved Pro187 demonstrated activity similar to Pro214, which does not contain a protease cleavable linker.

[0055] Figure 32 shows the TDCC activity of Format 2 construct Pro186, which uses n MMP9 cleavage site. Pro186 in the TDCC assay was 18-fold more active when added pre-cleaved than when added uncleaved. The pre-cleaved Pro186 demonstrated activity that fell between the positive controls Pro51 and Pro201. The uncleaved Pro186 demonstrated more activity than Pro214, which does not contain a protease cleavable linker.

[0056] Figure 33 depicts that the Pro186 construct binds to cells that have different levels of EGFR receptors, with CHO cells not expressing EGFR on the cell surface. Pro186 saturates cells expressing differing levels of EGFR at similar COBRA concentrations.

[0057] Figure 34 shows the schematics of Format 2 constructs used in the *in vivo* studies of Figure 35, all of which use sdABD-EGFR targeting domains.

[0058] Figure 35 shows that the Format 2 construct Pro186 is highly efficacious at both concentrations, and better than the Format 1 construct Pro140 at the lower concentration.

[0059] Figure 36 depicts a number of Format 2 constructs based on Pro186 but with different protease cleavage sites. While all of these constructs utilize sdABD-EGFRs for both targeting domains, other sdABDs to different TTAs can be used, and can be the same or different. That is, both homo-targeting (both targeting sdABDs to the same TTA) or hetero-targeting (one sdABD to a first TTA and the other to a different TTA) can be done.

[0060] Figure 37 depicts the schematics for different Format 2 constructs that vary linker length between the Fv domains. These are shown using an MMP9 cleavage site, although others can be used as outlined herein. Similarly, while all of these constructs utilize sdABD-EGFRs for both targeting domains, other sdABDs to different TTAs can be used, and can be the same or different.

[0061] Figure 38 shows that the linker length for the pseudo Fv can be varied, e.g. that a Format 2 construct with a short linker between the active Fv ("short active") with a longer linker between the pseudo Fv ("long inactive") exhibits similar activity to a "short active" with a "short inactive". Thus conditionality of the COBRA construct is not dependent on both the active and inactive scFv linkers being constrained; as long as one of them is constrained, single chain diabody folding appears to be favored over bivalent scFv folding.

[0062] Figure 39 shows that the linker length for the active Fv can be varied, e.g. that Format 2 constructs with "long active" and "short inactive" behaves similarly to a "short active" and "short inactive" construct. Thus conditionality of the COBRA construct is not dependent on both the active and inactive scFv linkers being constrained; as long as one of them is constrained, single chain diabody folding appears to be favored over bivalent scFv folding.

[0063] Figure 40A – Figure 40C shows the schematics for a number of different constructs. Pro188 is a Format 1 construct which is similar to Pro140 except with a long linker (16mer) in the pseudo Fv. Pro189 and Pro190 (Format 2 constructs) are similar to Pro186 and Pro187 except with a long linker (16mer) in the pseudo Fv domain. Pro191 and Pro192 (also Format 2 constructs) are similar to Pro189 and Pro190 except they have an additional cleavage site upstream of the sdABD(1/2). Pro193 (Format 4) has a single EGFR targeting domain, the iVH and iVL rearranged to be in reversed order, and an additional cleavage site upstream of the sdABD(1/2). Pro195 is a Format 2 construct similar to Pro186, with targeting domains that bind to the same TTA, EGFR, but to different epitopes. Pro196, Pro197 and Pro198 are Format 2 constructs with rearranged variable domains.

[0064] Figure 41 depicts the fact that different sdABD clones directed to human FOLR1 show differential killing. A Pro22 type construct (Pro51 with a FLAG sequence instead of a NCL) that binds to human FOLR1 was compared to a Pro22-EGFR construct against a number of cell line families.

[0065] Figure 42 depicts the schematics for four sdABD-FOLR1 constructs, including the use of the Pro201 positive control using sdABD-EGFR2 (with two molecules intermolecularly associating to form two active Fvs against CD3), and two Format 2 test articles, Pro311, using the h77.2 sdABD and Pro312 using the h59.3 sdABD, as well as two negative controls, Pro299, using the h77.2 sdABD and Pro303 using the h59.3 sdABD.

[0066] Figure 43 depicts the schematics of the Format 2 constructs for the FOLR/MMP9 in vivo design.

[0067] Figure 44 shows the efficacy of the Pro312 construct *in vivo*, and demonstrates the MMP9 cleavable linker is necessary for anti-tumor activity.

[0068] Figure 45 depicts the schematics of some formats using sdABDs to human B7H3 (sdABD-B7H3), including Pro244, the positive control (using sdABD-B7H3 (hF7) (with two molecules intermolecularly associating to form two active Fvs against CD3), and two Format 2 test articles, Pro225, a Format 2 construct, and Pro295, the negative control lacking a cleavage site.

[0069] Figure 46 shows that Pro225 has great conditionality as compared to the control, Pro295.

[0070] Figure 47 shows that a Format 2 construct of using a meprin linker, Pro373, shows great conditionality compared to Pro295.

[0071] Figure 48 depicts a number of sdABD-B7H3 (using the hF12 sequence) constructs, showing the Pro51 positive control using sdABD-EGFR, the Pro244 positive control using sdABD-hF12 B7H3, the test construct, Pro226, and the negative control Pro296 without a cleavage site.

[0072] Figure 49 shows the good conditionality of the Pro226 construct in a TDCC assay.

[0073] Figure 50 shows the humanization of sdABDs to human EpCAM.

[0074] Figure 51 shows the schematics of a number of Formats: Pro22hVIB13 and Pro205 are positive controls, Pro199 is a Format 2 construct and Pro175 is the negative control.

[0075] Figure 52 shows the TDCC activity of sdABD-EpCAM constructs, showing good conditionality.

[0076] Figure 53A – Figure 53B shows the TDCC activity of sdABD-EpCAM Pro199 construct, showing good conditionality in HT29 and LoVo cell models.

[0077] Figure 54A – Figure 54B shows the TDCC activity of sdABD-EpCAM Pro200 construct, showing good conditionality in HT29 and LoVo cell models.

[0078] Figure 55 shows a schematic of Pro255, which uses two different sdABD-TTA, one to EGFR (sdABD-EGFR) and the other to EpCAM (sdABD-EpCAM), as compared to Pro199, with dual EpCAM sdABDs. These are sometimes referred to herein as "hetero-targeting" constructs, in this case, a Format 2 construct.

[0079] Figure 56 shows that the Pro255 dual targeting molecule with an MMP9 cleavage site, shows good conditionality.

[0080] Figure 57A – Figure 57D shows the results of experiments on three different cell types. First, Raji transfectants were created with similar expression levels of EpCAM, EGFR and EpCAM + EGFR (data not shown). Then Pro255, which targets both EpCAM and EGFR, was tested in TDCC assays using each cell type. Figure 57A shows the parental Raji line,

that doesn't express either receptor. Figure 57B shows conditionality on the EpCAM line. Figure 57C shows conditionality on the EGRF line. Figure 57D shows conditionality on the EpCAM/EGFR line.

[0081] Figure 58 depicts the schematics of a Format 4 construct, Pro258.

[0082] Figure 59A – Figure 59B shows that Pro258 is conditional in both FBS and human serum. The conditionality of the MMP9 linker is underestimated due to the MMP9 activity in the culture. Interestingly, Pro51 TDCC activity is inhibited by HSA binding while Pro258 TDCC activity is similar to Pro51 in the presence of HSA. Finally, the Pro258 conditionality is somewhat enhanced in the presence of HSA by 6X.

[0083] Figure 60A – Figure 60C shows the cleavage of the MMP9 substrate by other MMPs.

[0084] Figure 61A– Figure 61B shows some of the exemplary constructs and their formats.

[0085] Figure 62A – Figure 62U shows a number of sequences of the invention, although many additional sequences are also found in the sequence listing. CDRs are underlined and bolded, linkers are double underlined (with cleavable linkers being italicized and double underlined) and domain separations are indicated by "/". All His6 tags are optional, as they can be used to reduce immunogenicity in humans as well as be purification tags.

VI. DETAILED DESCRIPTION OF THE INVENTION

A. Introduction

[0086] The present invention is directed to methods of reducing the toxicity and side effects of bispecific antibodies (including antibody-like functional proteins) that bind to important physiological targets such as CD3 and tumor antigens. Many antigen binding proteins, such as antibodies, can have significant off-target side effects, and thus there is a need to only activate the binding capabilities of a therapeutic molecule in the vicinity of the disease tissue, to avoid off-target interactions. Accordingly, the present invention is directed to multivalent conditionally effective ("MCE") proteins that have a number of functional protein domains. In general, one of these domains is an antigen binding domain (ABD) that will bind a target tumor antigen (TTA), and another is an ABD that will bind a T-cell antigen such as CD3 under certain conditions. Additionally, the MCE proteins also include one or more protease cleavage sites. That is, the therapeutic molecules are made in a "pro-drug"

CLAIMS

1. A polypeptide comprising, from N- to C-terminal:

a) a first single domain antigen binding domain (sdABD) that binds to a human tumor target antigen (TTA) (sdABD-TTA);

b) a first domain linker;

c) a constrained Fv domain comprising:

i) a first variable heavy domain comprising a VHCDR1, VHCDR2 and VHCDR3;

ii) a constrained non-cleavable linker (CNCL); and

iii) a first variable light domain comprising VLCDR1, VLCDR2 and VLCDR3, wherein the CNCL is positioned between the first variable heavy domain and the first variable light domain and prevents the first variable heavy domain and the first variable light domain from interacting to form an active Fv capable of binding CD3;

d) a second domain linker;

e) a second sdABD-TTA;

f) a cleavable linker (CL);

g) a pseudo Fv domain comprising:

i) a first pseudo variable light domain;

ii) a non-cleavable linker (NCL); and

iii) a first pseudo variable heavy domain;

h) a third domain linker; and

i) a third sdABD that binds to human serum albumin (HSA);

wherein said first variable heavy domain and said first variable light domain are capable of binding human CD3 but said constrained Fv domain does not bind CD3; and the polypeptide does not bind CD3 when the CL is intact.

2. A polypeptide comprising, from N- to C-terminal:

a) a first sdABD-TTA;

b) a first domain linker;

c) a constrained Fv domain comprising:

i) a first variable heavy domain comprising a VHCDR1, VHCDR2 and VHCDR3;

ii) a constrained cleavable linker (CCL); and

iii) a first variable light domain comprising VLCDR1, VLCDR2 and VLCDR3, wherein the CCL is positioned between the first variable heavy domain and the first variable light domain and prevents the first variable heavy domain and the first variable light domain from interacting to form an active Fv capable of binding CD3;

d) a second domain linker;

e) a second sdABD-TTA;

f) a cleavable linker (CL);

g) a pseudo Fv domain comprising:

i) a first pseudo variable heavy domain;

ii) a non-cleavable linker (NCL); and

iii) a first pseudo variable light domain;

h) a third domain linker; and

i) a third sdABD that binds to human serum albumin;

wherein said first variable heavy domain and said first variable light domain are capable of binding human CD3 but said constrained Fv domain does not bind CD3; and the polypeptide does not bind CD3 when the CL is intact.

3. A polypeptide according to any of claims 1 to 2 wherein said first variable heavy domain is N-terminal to said first variable light domain and said pseudo variable light domain is N-terminal to said pseudo variable heavy domain.

4. A polypeptide according to any of claims 1 to 2 wherein said first variable heavy domain is N-terminal to said first variable light domain and said pseudo variable heavy domain is N-terminal to said pseudo variable light domain.

5. A polypeptide according to any of claims 1 to 2 wherein said first variable light domain is N-terminal to said first variable heavy domain and said pseudo light variable light domain is N-terminal to said pseudo variable heavy domain.

6. A polypeptide according to any of claims 1 to 2 wherein said first variable light domain is N-terminal to said first variable heavy domain and said pseudo variable heavy domain is N-terminal to said pseudo variable light domain.

7. A polypeptide according to any of claims 1-6 wherein said first and second TTA is the same.

8. A polypeptide according to any of claims 1-6 wherein said first and second TTA are different.

9. A polypeptide according to any of claims 1-8 wherein said first and second TTA are selected from EGFR, EpCAM, FOLR1 and B7H3.

10. A polypeptide according to any of one of claims 1-9 wherein said first and second sdABD-TTAs are selected from the group consisting of SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:21, SEQ ID NO:25, SEQ ID NO:29; SEQ ID NO:33; SEQ ID NO:37 and SEQ ID NO:41.

11. A polypeptide according to any of one of claims 1 to 10 wherein said third sdABD has the amino acid sequence of SEQ ID NO:45.

12. A polypeptide according to claim 1 to 11 wherein said cleavable linker is cleaved by a human protease selected from the group consisting of MMP2, MMP9, Meprin A, Meprin B, Cathepsin S, Cathepsin K, Cathespin L, GranzymeB, uPA, Kallekriein7, matriptase and thrombin.

13. A polypeptide according to claim 1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 145, 147, 148, 149, 150, 151, 152, 183, 153, 169, 170, 154, 155, 156, 176, 181, 182, 146, 165, 166, 167, 168, 157, 158, 159, 160, 161, 162, and 163.

14. A nucleic acid encoding a polypeptide according to any of claims 1 to 13.

15. An expression vector comprising the nucleic acid of claim 14.

16. A host cell comprising the expression vector of claim 15.

17. A method of making a polypeptide comprising culturing the host cell of claim 16 under conditions wherein said polypeptide is expressed and recovering said polypeptide.

18. Use of a polypeptide of any of claims 1-13 in the manufacture of a medicament for the treatment of cancer.

19. A composition comprising:

a) a first polypeptide comprising, from N- to C-terminal:

i) a first sdABD-TTA;

ii) a first domain linker;

iii) a pseudo Fv domain comprising, from N- to C-terminal:

1) a variable heavy chain comprising a VHCDR1, VHCDR2 and VHCDR3;

2) a cleavable linker (CL); and

3) a first pseudo inactive variable light (iVL) domain comprising iVLCDR1, iVLCDR2 and iVLCDR3;

iv) a second domain linker;

v) a second sdABD that binds to human serum albumin;

b) a second polypeptide comprising, from N- to C-terminal:

i) a third sdABD that binds to a human tumor target antigen;

ii) a third domain linker;

iii) a pseudo Fv domain comprising, from N- to C-terminal:

1) a variable light chain comprising a VLCDR1, VLCDR2 and VLCDR3;

2) a cleavable linker (CL); and

3) a first pseudo inactive variable heavy (iVH) domain comprising iVHCDR1, iVHCDR2 and iVHCDR3;

iv) a fourth domain linker;

v) a fourth sdABD that binds to human serum albumin;

wherein

said first variable heavy domain and said first variable light domain are capable of binding human CD3 when associated; the polypeptide does not bind CD3 when the CL is intact; and

wherein said first and third sdABD are selected from the group consisting of SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:21, SEQ ID NO:25, SEQ ID NO:29; SEQ ID NO:33; SEQ ID NO:37 and SEQ ID NO:41.

20. A composition comprising:

a) a first polypeptide comprising, from N- to C-terminal:

i) a first sdABD-TTA;

ii) a first domain linker;

iii) a second sdABD-TTA;

iv) a second domain linker;

v) a pseudo Fv domain comprising, from N- to C-terminal:

1) a variable heavy chain comprising a VHCDR1, VHCDR2 and VHCDR3;

2) a cleavable linker (CL); and

3) a first pseudo inactive variable light (iVL) domain comprising iVLCDR1, iVLCDR2 and iVLCDR3;

vi) a third domain linker; and

vii) a sdABD-HSA;

b) a second polypeptide comprising, from N- to C-terminal:

i) a third sdABD-TTA;

ii) a fourth domain linker;

iii) a fourth sdABD-TTA;

iv) a fifth domain linker;

v) a pseudo Fv domain comprising, from N- to C-terminal:

1) a variable light chain comprising a VLCDR1, VLCDR2 and VLCDR3;

2) a cleavable linker; and

3) a first pseudo inactive variable heavy (iVH) domain comprising iVHCDR1, iVHCDR2 and iVHCDR3;

vi) a sixth domain linker;

vii) a sdABD-HSA;

wherein

said first variable heavy domain and said first variable light domain are capable of binding human CD3 when associated; and

the polypeptide does not bind CD3 when the CL is intact.

21. A polypeptide comprising, from N- to C-terminal:

a) a single domain antigen binding domain (sdABD) that binds to a human tumor target antigen (TTA) (sdABD-TTA);

b) a first domain linker;

c) a constrained Fv domain comprising:

i) a first variable heavy domain comprising a VHCDR1, VHCDR2 and VHCDR3;

ii) a constrained non-cleavable linker (CNCL); and

iii) a first variable light domain comprising VLCDR1, VLCDR2 and VLCDR3;

d) a cleavable linker (CL);

e) a second sdABD that binds to human serum albumin;

f) a second domain linker;

g) a constrained pseudo Fv domain comprising:

i) a first pseudo light variable domain;

ii) a non-cleavable linker (NCL); and

iii) a first pseudo heavy variable domain;

wherein said first variable heavy domain and said first variable light domain are capable of binding human CD3 but said constrained Fv domain does not bind CD3; and
the polypeptide does not bind CD3 when the CL is intact.